The Tie That Binds: Eicosanoids in Invertebrate Biology

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ABSTRACT Eicosanoids are oxygenated compounds formed from C20 polyunsaturated fatty acids by reactions involving at least 1 step of mono- or dioxygenase-dependent oxidation. These compounds have been detected in species representing all major animal taxa including numerous insect species. Although these compounds are best understood in human biology where they have immense clinical medicinal significance, they are now recognized as universally important to all life forms. Indeed, these compounds appear to have been recruited into roles as biological signaling moieties long before the origin of the Metazoa. During the ensuing evolutionary diversification of animals, eicosanoids have been commandeered into a plethora of biological roles, some of which are known only from invertebrates. In this review we provide a brief chemical overview of the diversity of eicosanoids that have been discovered and discuss a number of physiological, behavioral and ecological systems where these compounds have been shown to be of major importance. Our discussions are couched in terms of comparative biology, and where it is appropriate, include evolutionary speculations.

KEY WORDS reproduction, defense, host-parasite relationships, ecology, behavior

CERTAIN BIOCHEMICALS, SUCH as nucleic acids and proteins, are ubiquitous to the phenomenon known as life, and detailed comparative taxonomic studies of the similarities and differences of these compounds and their biological functions have provided a unifying theoretical construct at all levels of biology. The plethora of oxygenated products of certain C20 polyunsaturated fatty acids, known as eicosanoids, represent a similar group of biomolecules that are ubiquitous to life and which also provide a unifying theoretical construct from cell–cell to organism–organism signaling processes (Stanley-Samuelson 1994, Stanley and Howard 1998). Such sweeping constructs only make sense, of course, if they are tested across a large array of taxa representing the maximum number of phylogenetic units and are found to meaningfully explain physiological, biochemical and ecological processes at every level of biological organization. Although the earliest research on eicosanoids was carried out on humans, and the vast majority of the published research is still on humans, there has been an explosion of research in the last 30 yr on eicosanoids from bacteria, protozoa, fungi, algae, higher plants, invertebrates and vertebrates (recent major reviews include Gerwick 1993, Stanley-Samuelson 1994, Samuelson et al. 1995, Piomelli 1996, Rowley 1996, Rowley et al. 1998, Stanley and Howard 1998, Stanley 1999). Some of this research has simply documented the presence of these molecules, whereas others have reported on their biochemical, physiological, and ecological roles.

Professor Carl Schaefer, to whom this article is dedicated for his 25 yr of service as Editor of the *Annals of the Entomological Society of America*, is one of a few remaining representatives of the Renaissance model of a scholar. In both his systematic studies and his vast array of outside interests, he has perceived the world as an evolving network of interacting entities, whose Gestalt can only be understood by a consideration of the whole and not by just an examination of the parts. A complete appreciation of the unifying properties of eicosanoids can also only be achieved by unraveling the network of interacting intra- and interorganismic relationships in which they function, and then synthesizing the resulting oeuvre into unifying principles that apply across the spectrum of life (the "biological paradigm" of Stanley and Howard [1998]). Although we are not yet anywhere near to that point, substantial progress has been made in recent years. This review will provide an overview of some of that progress in 2 major areas of science that Professor Schaefer has long had an interest in, reproductive biology and ecology.

Eicosanoids are a bewildering array of oxygenated moieties derived from 3 physiologically essential 20-carbon polyunsaturated fatty acids (Stanley-Samuelson 1994). Fig. 1 contains a few examples of the major groups of eicosanoids that have been found to date. These include prostaglandins and their derivatives (1–6), leukotrienes (7), and hydroxyeicosatetraenoic acids (8, 9).

Eicosanoids in Reproductive Biology

The known roles of eicosanoids in the reproductive biology of invertebrates occur at 2 distinct levels of

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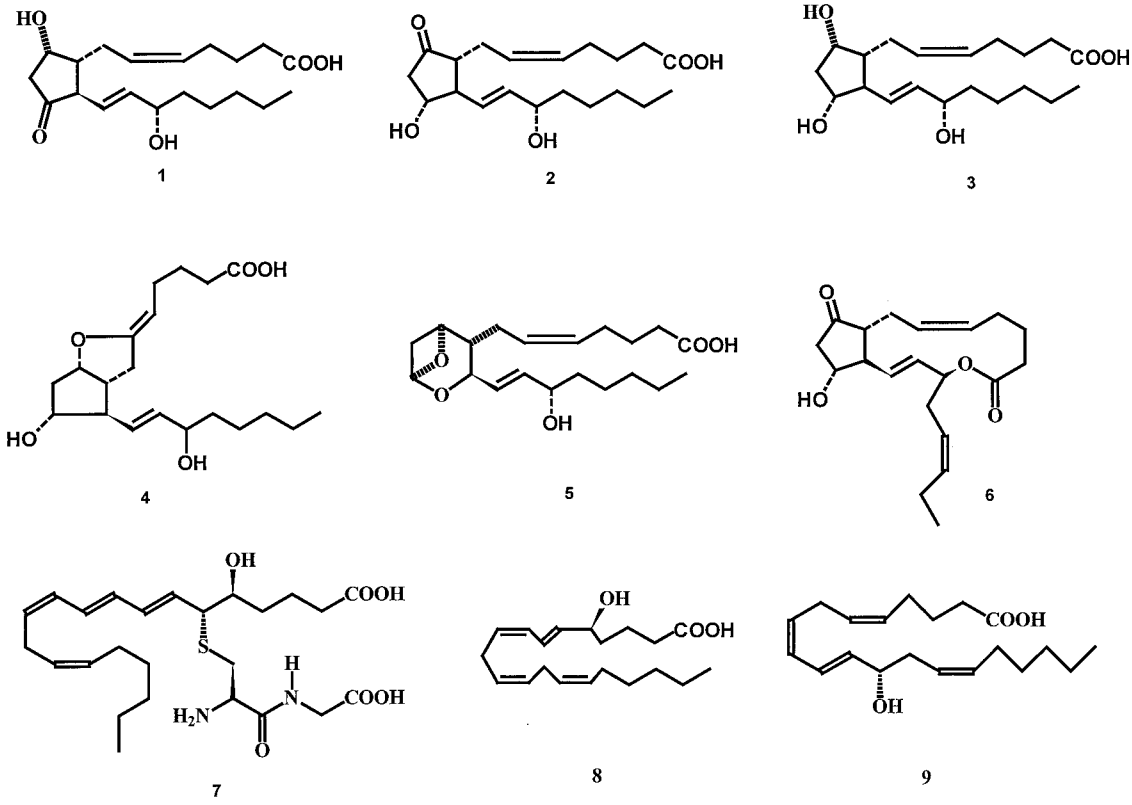

Fig. 1. Representative eicosanoids reported from invertebrate organisms. (1) PGD_2, (2) PGE_2, (3) $PGF_{2\alpha}$, (4) PGI_2, (5) Thromboxane A_2, (6) PGE_3-1,15-lactone, (7) Leukotriene D_4, (8) 5-(S)-hydroxyeicosatetraenoic acid, (9) 12-(S)-hydroxy-eicosatetraenoic acid.

biological organization, cellular and organismal. Historically, studies at the organismal level came 1st, led by the report of Destephano and Brady (1977) that the release of egg-laying behavior in newly mated house crickets, *Acheta domestica* (L.), was mediated by prostaglandins. The most detailed studies on the role of prostaglandins in releasing egg-laying behavior, however, came out of the laboratory of Werner Loher, who used as his experimental animal the Australian field cricket, *Teleogryllus commodus* (Walker). Loher and Edson (1973) showed that females were receptive to mating throughout their adult lives, commencing just hours after the adult molt, that their receptivity was not attenuated by successful copulation, and that males typically guarded their partners until after eggs were deposited. They further showed that mating stimulated egg production (both laid and stored) and that mated females deposited more eggs than virgins. The act of copulation alone, however, does not trigger increased oviposition. Males in which the testes were removed were shown to be attractive to females and capable of mating with them. Matings with such males did not, however, result in increased egg deposition. Loher and Edson (1973) concluded that a factor originating in testes stimulates egg production and release after transfer to females. At the time they did not know whether the factor was a chemical associated with the sperm mass or the sperm mass per se.

Loher (1979) and Loher et al. (1981) rigorously tested the hypothesis that prostaglandins release egg-laying behavior in gravid *T. commodus.* These studies included developing methods to measure in situ quantities of prostaglandins in spermathecae from virgin and mated females. Using pools of 100 spermathecae they found ≈500 pg of PGE_2/spermathecae in tissues from mated females and no PGE_2 in preparations from virgins. Inasmuch as the efficiency of extraction of these compounds was not reported, these values are best taken as estimates. The next question addressed by Loher and his group was where did this PGE_2 originate? Prostaglandins occur in mammalian semen in impressive amounts, and hence 1 possibility was that the males were transferring the PGE_2 in their seminal fluid. Analysis of spermatophores revealed only 20 ng PGE_2/spermatophore, a value ≈25-fold less than that found in the spermathecae, suggesting that the males are not transferring biologically meaningful levels of the prostaglandins in their seminal fluid. An alternative hypothesis was that the males were transferring the enzymes required for prostaglandin biosynthesis. To test this, fluids from the spermathecae and spermatophore were incubated with radioactive arachi-

donic acid (the requisite fatty acid required for the biosynthesis of PGE_2) and radioactive prostaglandins were isolated and counted by liquid scintillation. Virtually no prostaglandin biosynthetic activity was found in the spermathecal fluids from virgin females, whereas substantial activity was associated with spermathecae from mated females and from spermatophores (Loher et al. 1981). Thus, the males are indeed transferring the requisite enzymes to the female while mating, along with other seminal fluid components. The major additional component of interest is arachidonic acid, because this could be provided by the virgin female or by the male. The total fatty acid content of spermathecae of virgin females was shown to consist of almost 2% of arachidonic acid, whereas the content of arachidonic acid in mated females was almost 40 times as great. This difference was shown to be the contribution of the male (Stanley-Samuelson and Loher 1983).

Tobe and Loher (1983) further characterized the biosynthetic activity of *T. commodus* spermatophores and showed that both PGE_2 and $PGF_{2\alpha}$ were produced, with the former always present in greater quantities than the latter. Prostaglandin biosynthesis was shown to be linear with number of spermatophores used, sensitive to substrate concentration, and exhibited a pH optimum between pH 7.5 and 8.0. Using testectomized males, they also showed that testes were required to produce spermatophores with biosynthetic activity. Finally, the authors showed that incubations of the spermatophores in the presence of a known prostaglandin biosynthesis inhibitor (aspirin) resulted in a diminution of biosynthetic activity. Furthermore, they provided additional evidence on the identity of their product. The PGE_2 produced could be converted with 0.5 M NaOH into the known metabolite PGB_2, and large scale biosynthesis of PGE_2 and $PGF_{2\alpha}$ resulted in products which were detectable by high performance liquid chromatography and that co-eluted with known standards.

Loher in 1979 directly tested whether injected PGE_2 would stimulate egg-laying behavior in virgin *T. commodus.* His initial studies involved 50-μg injections of PGE_2 into the hemocoel, whereas his later studies (Loher et al. 1981) involved direct treatment of the genital chamber with nanogram levels of PGE_2. In both cases, strong egg-laying behavior by the virgin females was obtained. Although it might be argued that these are rather high levels of prostaglandins, Stanley-Samuelson and Loher (1985) showed that injected prostaglandins are rapidly removed from circulation and excreted along the usual insect pathways via the Malpighian tubule/hindgut complex and that such quantities are necessary to achieve physiologically meaningful titers. Stanley-Samuelson et al. (1986) also examined the effects of 17 different eicosanoids and eicosanoid metabolites known from the mammalian literature on the egg-laying behavior of *T. commodus.* The highest egg-laying activity was found with compounds sharing the ring structures of PGE_2, thus suggesting that prostaglandins of the E-series were the natural compounds responsible for egg-laying behavior. Surprisingly, however, the single most active compound was 15-keto-PGE_2, a biologically inactive catabolite in mammalian metabolism. Furthermore, compounds featuring additional oxygen atoms, including thromboxane B_2 and 15-keto-$PGF_{2\alpha}$ also stimulated high egg laying activity. The significance of these findings remains unknown.

The information from *T. commodus* very strongly supports the notion that prostaglandins are involved in regulating egg-laying behavior in this cricket species. All available data support the enzyme transfer model (Loher et al. 1981, Stanley-Samuelson and Loher 1986, Stanley-Samuelson 1994) wherein the male transfers the prostaglandin synthase complex and fatty acid substrate to the female via a spermatophore. Once within the females' spermathecae, PGE_2 is formed and released into hemolymph circulation, probably from the female common genital chamber. The PGE_2 then releases the egg laying behavioral program, probably by interacting with specific cell receptors located in the last abdominal segment.

How general is this model for eicosanoid actions on egg laying behavior in insects? Egg-laying behavior is under a wide variety of constraints and influences when considered across all of the Insecta, and one should anticipate that eicosanoids might release oviposition behavior in some insect species, but certainly not in all of them. The evidence to date supports this view. Among crickets, prostaglandins release egg-laying behavior in the house cricket *Acheta domesticus* (Destephano and Brady 1977), the field cricket *T. commodus* (Loher et al. 1981), the related cricket *T. oceanicus* (Le Guillou) (Vaughan 1995), and in *Gryllus bimaculatus* de Geer (Ai and Ishii 1984). The short tailed cricket *Anurogryllus muticus* de Geer (Lee and Loher 1995), however, does not use prostaglandins to release egg-laying, a finding in agreement with the mating system of this cricket where egg-laying behavior immediately follows mating. Beyond these few Orthoptera, evidence for the distribution and frequency of prostaglandin or other eicosanoid mediated egg-laying behaviors is sparse [a moth, *Bombyx mori* (L.) (Yamaja Setty and Ramaiah 1979, 1980); a beetle, the twenty-eight spotted ladybird *Henosepilachna vigintioctopunctata* F. (Izawa et al. 1986); a hemipteran, the brown rice planthopper *Nilaparvata lugens* Stål (Uchida et al. 1987); and the firebrat *Thermobia domestica* (Packard) (which uses hydroxyeicosatetraenoic acids rather than prostaglandins) (Rageb et al. 1987, 1991, 1992; Bitsch et al. 1995)]. Although these insects represent several of the major insect orders, we do not think it likely that prostaglandins will be shown to mediate egg laying in the majority of insects. Indeed, there are several insect species in which prostaglandin titers increase in female reproductive tracts after mating, but no evidence suggests that this increase is related to egg-laying [the cabbage looper, *Trichoplusia ni* (Hübner) (Hagan and Brady 1982); the desert locust *Locusta migratoria* L. (Lange 1984), and houseflies, *Musca domestica* L. (Wakayama et al. 1986)].

Table 1. Survey of known marine invertebrates that use eicosanoids to regulate their reproductive processes

Taxon	Eicosanoid type	Eicosanoid role	References
Barnacles	mono- and trihydroxy-tetraenoic acids	Egg hatching	Clare et al. 1982, 1985; Hill and Holland, 1992; Hill et al. 1998, 1993; Holland et al. 1985; Song et al. 1990a, 1990b
Scallops	PGE_2 and $PGF_{2\alpha}$	Spawning & egg release	Matsutani & Nomura 1986, 1987; Osada et al. 1989
Crayfish	PGE_2 and $PGF_{2\alpha}$	Vitellogenesis, induction of ovulation	Spaziani et al. 1993, 1995
Prawns	PGE_2	Vitellogenesis	Sagi et al. 1995
Snails	Prostaglandins	Egg production, egg mass deposition	Clare et al. 1986; Kunigelis and Saleuddin 1986; van Duivenboden 1983
Red Abalone	Prostaglandins	Spawning	Morse et al. 1977
Starfish	Lipoxygenase products; 8-R-hydroxy-eicosatetraenoic acid	Oocyte maturation	Meijer and Mordret 1994; Meijer et al. 1984, 1986a, 1986b, 1986c
Sand dollars	Leukotriene B_4	Intracellular calcium release from eggs	Silver et al. 1994
Sea urchins	Prostaglandins and lipoxygenase products	Prevention of polyspermic fertilization	Hawkins and Brash 1987, 1989; Perry and Epel 1985a, 1985b; Schuel et al. 1984, 1985

An important point is that, even where eicosanoids mediate organismal events, they likely do so by mediating actions on cells in the central nervous system of the organism. Consider, for example, the question of how prostaglandins release egg-laying behavior in the various crickets. Initially it was thought that, in analogy to mammalian systems, the prostaglandins might act by stimulating contractions of the oviduct muscles, thus moving eggs through the reproductive tract during oviposition. However, Loher (1984) showed that PGE_2 does not stimulate contractions in the *T. commodus* oviduct muscles and Cook et al. (1984) showed that PGE_2 did not stimulate the oviduct muscles of the cockroach *Leucophaea maderae* (F.). Loher and his colleagues (Ai et al. 1986, Stanley-Samuelson and Loher 1986) therefore analyzed the entire oviposition program of *T. commodus*, which consists of several discrete elements, including assessing the quality of potential egg deposition substrates, probing the substrate, extruding an egg, and withdrawing the ovipositor. They showed that this suite of individual behavioral programs resides in nerve networks within the last abdominal ganglion, and that these networks interact with the central nervous system to control the complete behavior. Somehow, eicosanoids must act as messengers in this scenario, although the individual steps remain obscure.

At a lower level of organization, more is known about how prostaglandins act to provide an egg-laying signal in *T. commodus.* PGE_2 quantities increase in the spermathecae and hemolymph after mating (Loher et al. 1981, Ai et al. 1986) as suggested by the enzyme transfer model, and hence one could argue that the egg-laying signal may be simply an increase in circulating PGE_2. But along with the PGE_2 increases, even greater decreases in spermathecal and hemolymph titers of $PGF_{2\alpha}$ occur (Stanley-Samuelson et al. 1983, Ai et al. 1986). Calculated with respect to each other, spermathecael quantities of these 2 prostaglandins change by almost 1,755-fold between virgin and mated females, and perhaps this change is the specific egg-laying signal in newly mated females. The exact site at which these changes in prostaglandin titer are registered is not known, but Loher (1984) has suggested that the central nervous system is a prime candidate.

Additional insights into the role of eicosanoids in invertebrate reproductive systems have come from numerous studies of fresh water and marine organisms, where the studies to date have focused mainly on cellular events rather than organismal ones (Table 1). These cellular events, such as vitellogenesis, oocyte maturation, prevention of polyspermic fertilization, almost certainly have their counterparts in terrestrial arthropods and other land invertebrates, and hence these studies are important as guidelines for further comparative research. Indeed, such studies, cited in Table 1, led to the "biological paradigm" proposed in Stanley and Howard (1998).

Ecological Roles: Predator–Prey Interactions

It is becoming increasingly clear that eicosanoids also mediate a variety of ecological interactions. We selectively review here the invertebrate literature dealing with the roles of eicosanoids in predator-prey interactions, the roles of eicosanoids in host-parasite interactions, and the influence of insect-derived eicosanoid biosynthesis inhibitors in ecological interactions. In every case, the implications for other levels of ecology are obvious and we have no doubt that as time goes by, appreciation of eicosanoids in chemical ecology will substantially increase.

The first discovery of eicosanoids in an invertebrate was in the octacoral, *Plexaura homomalla* (Esper) (Weinheimer and Spraggins 1969), and it elicited quite a bit of excitement, both because synthetic eicosanoids were not readily available at that time and because the concentration of prostaglandins in this coral species was so high (8% of wet tissue weight!). Further studies revealed that only a few species of octacorals possessed these high levels of prostaglandins, and the inference was drawn that they must have some adaptative value. Gerhart (1984) was the first to suggest that elevated prostaglandin levels in these corals might serve as a chemical defense against predation by coral-eating fish. Prostaglandins induce

vomiting (emesis) in many vertebrates, including humans, when delivered into the stomach by direct feeding. The crux of Gerhart's hypothesis is that fish and other vertebrate predators are capable of developing a learned aversion to emetic foods. In this model, at least some species of coral-eating fish might eat a portion of the high prostaglandin containing tissue, experience a bout of vomiting, and thereafter avoid the emetic coral. Gerhart (1991) tested this hypothesis in a series of laboratory and field experiments, using PGA_2 and its 15-R epimer (the naturally occurring prostaglandins in the corals) applied to bait pieces of cooked fish muscle from several species of fish. These emetic food pellets were offered to a variety of fish species. As predicted, the test fish species initially accepted emetic and control baits at identical rates. But by the third time the fish were offered the choices, they consistently rejected all emetic baits and accepted only control baits. The major caveat to these studies is that none of the bioassay fish used were natural members of the coral community, and none of the experiments used pieces of coral as food items. The author's conclusions are eminently reasonable, however, and in all likelihood true.

Nudibranchs are gastropod molluscs, most of which do not have a protective shell. As such, they might be thought to be highly exposed to fish predation. In contrast to this expectation, the often brightly colored nudibranchs are rather well protected from fish by a variety of defensive ploys. They possess structures known as cerata, readily lost from their body when attacked by predators. Detached cerata produce and exude a defensive slime over several hours. This slime contains, among other allelochemicals, a group of novel prostaglandin 1,15-lactones. Cimino et al. (1989, 1991a, b) were the first to discover these chemicals, and lactone derivatives of PGE_2, PGA_2, and $PGF_{2\alpha}$ have been characterized. In addition, lactone-11-acetates and carbon-9 and carbon-11 fatty acid esters of the lactones have been found in nudibranchs. In addition to the autonomous cerata, the nudibranchs have a mantle that produces high concentrations of free prostaglandins, and their corresponding lactones for transport to the cerata (Di Marzo et al. 1991). These authors suggested that the lactones may serve a number of biological roles, including acting as a toxic alleochemical in the cerata mucus and as a source of inactive prostaglandin precursors that could be activated to produce emetic prostaglandins.

Marin et al. (1991) directly tested the hypothesis that the prostaglandin 1,15-lactones could serve defensive roles in the nudibranchs. They tested the toxicity of 7 different eicosanoids to mosquito fish, and found that PGE_3, PGE_2, and $PGF_{3\alpha}$ were nontoxic. In contrast, PGE_3-lactone was toxic at 1.0 μg/ml and PGE_2-lactone, PGA_3-lactone, and PGA_2-lactone were toxic at 10 μg/ml. It would seem that the nudibranchs have taken the defensive strategy of the octacorals 1 step further. Not only do they possess the emetic prostaglandins, they possess and apparently use, the prostaglandin 1,15-lactones as toxins. Thus, fish that are not killed outright by the toxins would presumably experience the learned aversion to the emetic prostaglandins and henceforth avoid eating similar appearing nudibranchs. Although these speculations need to be tested in the field, they are reasonable speculations that generate further testable hypotheses.

Ecological Roles: Host–Parasite Interactions

Invertebrate parasites have evolved some of the most intricate and complex life cycles known. Increasingly it is being recognized that many aspects of these complex host-parasite interactions are mediated by chemicals, including various eicosanoids. The early studies focused on demonstrating the presence of eicosanoids and the corresponding eicosanoid-biosynthetic enzyme complexes in the parasites, but as the field has grown in maturity, studies have begun to focus on the biological roles of the eicosanoids in the host-parasite interactions. It is the latter area that we will review, although it must be realized that it is critical to demonstrate that the parasite is indeed capable of producing and releasing the eicosanoids that are claimed to produce any given biological response.

Approximately 20,000 living species of flatworms (Phylum Platyhelminthes) are known, and many of them are important parasites of humans and domestic food animals. Eicosanoids appear to play vital roles in the life cycles of these parasites, including tapeworms (Class Cestoda) and flukes (Class Trematoda). Leid and McConnell (1983a, b) were the first to show that tapeworms could biosynthesize eicosanoids, specifically thromboxane A_2, PGE_2, and PGI_2. In these papers they set forth the hypothesis that the tapeworms were using the eicosanoids to attenuate the host defense reactions to the presence of a foreign invader. In particular, they hypothesized that the PGE_2 attenuated host immune responses and that the PGI_2 served to inhibit clotting mechanisms in the vicinity of the tapeworms.

Salafsky and his colleagues (Fusco et al. 1985, 1986, 1988, 1993; Salafsky and Fusco 1987; Salafsky et al. 1984a, b) have conducted a lengthy series of experiments on the role of eicosanoids in the relationship between the cercarial stage of the endoparasitic blood fluke, *Schistosoma mansoni* (Sombon) (a trematode), and its mammalian hosts. The life cycles of trematodes usually includes at least 2 hosts. In *Schistosoma*, eggs are produced by adult flukes in their hosts. The eggs are excreted in the host's feces and if they land in water, the eggs develop into a larval form known as miracidia. These miracidia then penetrate into the intermediate host, an aquatic snail in the case of *S. mansoni.* The larvae then develop through several asexual generations and emerge from the snail as free swimming larvae known as cercariae, which, given the opportunity, penetrate the skin of humans who may be present in the water.

Salafsky et al. (1984a) demonstrated that the cercariae were attracted to human skin lipids, particularly the polyunsaturated essential fatty acids, including arachidonic acid, the precursor to prostaglandins and other eicosanoids. They showed that the larvae moved

to the source of the polyunsaturated acids, and then stopped there. In their next series of experiments, Salafsky et al. (1984b) considered the influence of these acids (and their potential eicosanoid metabolites) on the ability of the cercariae to penetrate the skin and enter the host where they then typically shed their "tail" and become a form known as schistosomules. These experiments showed that the essential fatty acids enhanced cercarial penetration, and that prostaglandins were involved as important cellular signals in the process. In these sets of experiments, the authors were not able to unequivocally assess whether the prostaglandins came from the host or the tapeworm, although their finding that incubating the cercariae with prostaglandin biosynthesis inhibitors reduced the cercarial penetration rate by more than 80% strongly suggests that the tapeworms make their own eicosanoids. Fusco et al. (1985) settled the point with radioactive studies that demonstrated the capability of the cercariae to biosynthesize PGE_2, PGD_2, leukotrienes B_4 and C_4, and 5- and 15-hydroxyeicosatetraenoic acid. In further studies, Fusco et al. (1986) found that the lipoxygenase products were highly correlated with penetration behavior and that the cyclooxygenase products were correlated with the transformation of the cercariae to the schistosomulae form. They also postulated that the eicosanoids might be involved in host defense reactions. They further considered that some of the eicosanoids produced by the tapeworms were regulators of their own internal metabolism, whereas others were released into host tissues. Salafsky and Fusco (1987) tested this by determining the levels of internal and released radioactive eicosanoids in developing schistosomulae and adults, the forms of *S. mansoni* living in mammalian hosts.

Both the schistosomulae and the adults secreted eicosanoids into their surrounding medium. The schistosomulae released ≈64% of the total radioactive eicosanoids they produced, whereas adult males released 84% and adult females 86%. The eicosanoids released by the schistosomulae consisted of ≈63% of the total prostaglandins, 75% of the leukotrienes, and 58% of the hydroxyeicosatetraenoic acids. Adult males and females secreted roughly equal proportions of the total radioactive eicosanoids, a little over 80% of the prostaglandins and leukotrienes, and ≈90% of the hydroxyeicosatetraenoic acids. Salafsky and Fusco (1987) also determined total eicosanoid production in developing schistosomulae and adults by radioimmunoassay. The adults produced far more immunoreactive products than did the developing schistosomulae. They interpreted these findings in terms of the potential immunosuppresive roles of eicosanoids secreted from the blood flukes. Of course, mammalian skin is competent in its own right to mount immunological responses to infectious and parasitic agents, although no such reactions have been reported with respect to cercarial penetration. Fusco et al. (1988, 1993) therefore postulated that cercarial eicosanoids inhibit host skin immune reactions and conducted several complicated experiments to test this notion. The authors concluded from these studies that cercariae are able to influence some elements of mammalian skin reactions to invasions. They showed that cercariae increased production and release of interleukin-1 and down-regulated the release of the proinflammatory compounds interleukin-8 and leukotriene B_4. The down-regulation of these 2 compounds would presumably attenuate the host-defense activities of neutrophils, macrophages and T-cells. Fusco et al. (1993) also noted that other proinflammatory compounds were not up-regulated in the presence of cercariae. The authors recognized, of course, that mammalian immune responses are modulated and regulated by many compounds other than eicosanoids, including peptides, biogenic amines, and cytokines. It would not be surprising, therefore, if the invading parasites did not impact some of these other immunity mediating chemicals.

More recently, Baset et al. (1995) characterized the eicosanoid biosynthesis systems of adult, but not larval, *S. mansoni.* They found evidence for the production of a single lipoxygenase product, 15-hydroxyeicosatetraenoic acid and only the slightest evidence for cyclooxygenase products. As yet they have not extended this work to address the physiological roles of this product in the survivorship of the adult blood flukes.

Salafsky et al. (1990) also investigated the biology and ecology of eicosanoids in a nematode, the human hookworm, *Necator americanus* (Stiles). The adults of these animals attach themselves to the mucosa of the intestinal tract, and the eggs from the attached adults are deposited on the soil along with the host feces. Larvae hatch and then go through 2 molts before reaching the infective 3rd larval stage. This stage enters its human host by penetrating the skin, and in this regard, *N. americanus* is ecologically similar to *S. mansoni.* The larvae migrate through the skin and make their way to the lungs via blood or lymph circulation. The larvae molt into their 4th stage in the lungs, from where they migrate to the esophagus and become established by way of the tracheae.

As with *S. mansoni*, the hookworm larvae are attracted to human skin lipids and the free fatty acid fraction significantly increases larval penetration rates. The hookworm larvae also biosynthesize several eicosanoids (PGE_1, PGE_2, leukotrienes B_4 and C_4, and 15-hydroxy-eicosatetraeneoic acid) (Salafsky et al. 1990). By analyzing the parasites and their medium separately, it was shown that virtually all of the eicosanoids were secreted into the medium. As with *S. mansoni*, the hookworm larvae undergo morphological changes immediately after host penetration. Although the larvae of both species remain for hours at the epidermal-dermal junction before moving into the dermis, hookworms cause a classic proinflammatory reaction in the skin, whereas the cercariae do not. Salafsky et al. (1990) concluded by hypothesizing that the hookworm larvae also modulate direct immune responses of their hosts in a manner that is advantageous to them, but no direct experimental tests were conducted to test this hypothesis.

Lymphatic filariasis is a parasitic disease caused by several nematode species, including *Wuchereria ban-*

crofti (Cobbold) and *Brugia malayi* (Brug). Adults of these nematodes live in the lymphatic vessels of humans and other vertebrates. The adults give rise to larval forms known as microfilariae, which are found in the blood stream and migrate into the peripheral circulation on a daily basis corresponding to the host seeking periods of female mosquitoes. The microfilariae are then ingested with the blood meal of a mosquito. They then move through the mosquitoes, passing the midgut integument into the hemolymph, from there to the muscles and ultimately to the mosquito salivary glands, from whence they are injected into another human host while the infected mosquito is blood feeding.

Microfilariae seem to be mostly protected from the immune responses of their human hosts and generate few parasite-specific antigens. Weller and his colleagues postulated that this is because the microfilariae release eicosanoids into their hosts, similar to that noted with the tapeworms discussed above. Their studies used small rodents known as jirds (gerbils), which were infected with microfilariae of *B. malayi.* Liu et al. (1990) reported that the microfilariae produced PGD_2, PGE_2, and 6-keto-$PGF_{1\alpha}$, the stable end product derived from PGI_2. In the presence of eicosanoid biosynthesis inhibitors, the productions of these cyclooxygenase products were severely curtailed. The biosynthesis of PGI_2 was considered quite meaningful, because this compound potently inhibits platelet aggregation in mammals. Thromboxanes, which enhance platelet aggregation, were not produced by the parasites. PGE_2 and PGI_2 are both also vasodilators, and PGE_2 suppresses several proinflammatory actions, including granulocyte and macrophage functions, T-lymphocyte activation and lymphokine biosynthesis and release in mammals. Not surprisingly, Liu et al. (1990) suggested that the release of PGE_2 and PGI_2 might help the microfilariae manipulate the immune reactions of their hosts.

Liu and Weller (1992) tested this idea more directly by assessing the ability of microfilariae to inhibit platelet aggregation. The microfilariae inhibited platelet aggregation in a dose-dependent manner. The authors also showed that the microfilariae inhibited 2 other platelet activities: inhibition of thromboxane A_2 biosynthesis and release of serotonin. These 3 actions together severely compromised normal platelet function. Liu and Weller (1992) further demonstrated that the microfilariae did not require direct physical contact with the platelets to produce their effects, but rather produced soluble factors that could move across cell membranes to effect their action. These soluble factors were shown to be the prostaglandins they secreted. Clearly, it is of vital importance to the parasites that they are able to move freely through the microcirculation in the periphery of their host to be available for ingestion when mosquito vectors take a meal. Indeed, mosquitoes are more than just vectors, they are also intermediate hosts in which the nematodes must pass through several developmental phases. It would appear that the eicosanoids the microfilariae release into their vertebrate hosts is vital to this process.

The role of eicosanoids in the biology and ecology of the larval stages of another nematode parasite, *Oesophagostonum dentatum* (Ruldolphi), a nodular worm of pigs, has been extensively investigated by Daugschies and his associates (Daugschies 1995, 1996; Daugschies and Ruttkowski 1998). Third stage larvae biosynthesize and secrete 4 cyclooxygenase products (PGE_2, PGD_2, $PGF_{2\alpha}$, and thromboxane B_2) (Daugschies 1995), as well as leukotrienes (Daugschies 1996). In further studies, 3rd stage larvae cultured in the presence of cyclooxygenase and lipoxygenase inhibitors, have lengthened developmental times and reduced growth rates in transforming to 4th stage larvae. In the presence of indomethacin, a potent cyclooxygenase inhibitor, development to the 4th stage was completely inhibited. These effects on development were reversed by removing the inhibitors. Furthermore, Daugschies and Ruttkowski (1998) found that exposure of the larvae to the eicosanoid inhibitors arrested their ability to migrate out of an agar media, and that this inhibition also could be reversed by removing the inhibitors. Daugschies thus reasonably suggested that the eicosanoids produced by these nematode larvae were involved in normal developmental and ecological processes (larval migration), and he further suggested that because the eicosanoids were released into the culture media, they also are involved in attenuating the host immune response in the same manner as described above for the 3 lymphatic filariasis nematode parasites. As of yet his group has not made any direct tests of this latter hypothesis.

Another disease of global significance is malaria. One causative agent, the protozoan *Plasmodium falciparum* Welch, is transmitted by mosquitos. Kubata et al. (1998) have shown that this protozoa produces and releases into its environment PGD_2, PGE_2, and $PGF_{2\alpha}$. The prostaglandin production was increased when the protozoa were cultured in the presence of arachidonic acid, but addition of 2 mammalian cyclooxygenase inhibitors, aspirin or indomethacin, did not reduce prostaglandin production. These authors further showed that PGD_2 and PGE_2 accumulated in the culture medium mainly at the stage of trophozoites and schizonts. They speculate that these prostaglandins may be involved in the clinical manifestation of malaria.

The examples above relate to the roles of eicosanoids in interactions of endoparasites with their hosts. Eicosanoids serve similar functions in the interactions of ectoparasites with their hosts. All ticks are obligate ectoparasites, feeding on blood meals derived from their hosts. Unlike hematophagous insects, which take small blood meals in a short period, ticks remain on their hosts through an entire developmental stadium, during which they continuously ingest blood. Tick salivary glands are multifunctional organs, producing copious saliva, cement for attachment to their hosts, and other pharmacologically active materials that facilitate tick blood feeding. It is now apparent that eicosanoids are the important pharmacologically active components in tick saliva (Dickinson et al. 1976, Higgs et al. 1976, Shemesh et al. 1979, Ribeiro 1987). Four species of ticks have been examined for

the presence of eicosanoids: *Boophilus microplus* (Canestrini), *Hyalomma anatolicum* Koch, *Ixodes dammini* Say, and *Amblyomma americanum* (L.). Only cyclooxygenase products have been detected: PGE_2 in *B. microplus* (Higgs et al. 1976), PGE_2 and $PGF_{2\alpha}$ in *H. anatolicum* (Shemesh et al. 1979), PGE_2 and 6-keto-$PGF_{1\alpha}$ (the stable metabolic product of PGI_2) in *I. dammini* (Ribeiro et al. 1988), and PGE_2, $PGF_{2\alpha}$, PGD_2, and PGA_2/PGB_2 in *A. americanum* (Ribeiro et al. 1992; Bowman et al. 1995; Pedibhotla et al. 1995, 1997).

Dickinson et al. (1976) and Shemesh et al. (1979) both suggested that the ticks might secrete prostaglandins to increase vascular permeability in the region of the feeding lesion. Higgs et al. (1976) suggested that the PGE_2 present in the tick saliva could be important in starting and maintaining the feeding lesion of the ticks. Ribeiro et al. (1988) interpreted their finding of PGI_2 in the saliva of *I. dammini* as a mechanism of sustaining blood flow around the feeding wound by preventing platelet aggregation. The presence of this eicosanoid could also prevent mast cell degranulation, which would minimize host reaction to the ticks. Ribeiro et al. (1988) showed that the tick saliva contained more than enough of these eicosanoids to be pharmacologically significant under real world conditions. Sauer et al. (1993) summarized the major arguments for the roles of prostaglandins as immunosuppresive agents in mammalian hosts. Tick saliva attenuates host competence in several ways. Homogenates of salivary glands suppress activation of lymphocytes and the release of cytokines. PGE_2 prevents macrophage activation and neutrophil activity and promotes vasodilation. PGI_2 inhibits cellular immune reactions, including mast cell degranulation. These data, along with the vast literature on tick behavior, strongly support the hypothesis that prostaglandins associated with tick saliva are very important agents in tick-host relationships.

Ecological Roles: Insect-Derived Eicosanoid Biosynthesis Inhibitors

Twelve years ago, Howard et al. (1986) reported that the defensive secretion of the red flour beetle, *Tribolium castaneum* (Herbst), contained substantial quantities (>0.25% of the total wet biomass) of 2 aromatic β-hydroxyketones that are potent inhibitors of prostaglandin H synthase ($\approx$100 times more potent than the classical inhibitor of this enzyme, aspirin). Examination of several other *Tribolium* species showed that the presence of the β-hydroxyketones and related aromatic β-hydroxyesters (Howard and Mueller 1987) were distributed in a phylogenetic manner, with some subgenera not containing any at all (Howard 1987). Howard and his colleagues extended their search for similar prostaglandin H synthase inhibitors to other insect defensive secretions, and reported that insects in several different orders contained substantial quantities (usually >0.1% of the total biomass of the insect) of this class of chemicals (Jurenka et al. 1986, 1989). In every case the pertinent components were present in mixtures of other compounds that were genuinely noxious in 1 manner or another. The eicosanoid biosynthesis inhibitors, in contrast, exhibited no apparent noxious properties.

What might be the ecological or physiological meaning of the production of these eicosanoid biosynthesis inhibitors in such a diverse array of insect species? Eicosanoids appear to act in 2 major areas of animal physiology. One might be termed the "housekeeping arena" in which the eicosanoids act to variously influence cellular actions necessary for maintaining organismal homeostasis. The other major arena relates to eicosanoid actions at crucial stages in the life history of an organism. Some of these actions, perhaps at a key point in development or reproduction, may occur only once in the life history. The roles of certain lipoxygenase products in oocyte maturation in starfish and barnacle hatching are examples. Other crucial points in a life history, such as cellular reactions to bacterial infections or wounding may occur relatively frequently. Temporary inhibition of eicosanoid actions in the housekeeping arena would probably not result in lasting damage to most organisms. If, however, eicosanoid biosynthesis was inhibited at a crucial point, say at the early phases of a bacterial infection or during the penetration phase of a parasitization process, then the effects of the inhibition could be highly deleterious to the organism. These speculations must remain just that until the necessary behavioral and ecological experiments are conducted.

Eicosanoids have now been found in virtually every life form taken under appropriate scrutiny. Such ubiquity implies that these molecules serve vital functions, and that they have done so over most of evolutionary time. We suggest that these molecules were drawn into various roles as signal transduction moieties very early in the history of cellular life. As these early cellular life forms evolved into more complex metazoan organisms, individual cells were preadapted to use eicosanoids as modulators of events. The number and variety of eicosanoid roles continued to increase throughout all stages of evolution, until we reached the present day plethora of eicosanoids and roles. As we reach a better understanding of these various eicosanoid roles in an ever-increasing spectrum of life forms, our understanding of many other biological phenomena is facilitated. Indeed, it has become apparent that in agreement with the diversity of life forms, there is an equally diverse collection of eicosanoids and functional roles for those eicosanoids. Although it is tempting to overlay these many eicosanoids and roles over our current understanding of phylogenetic relationships, not enough data have been gathered to confidently do so. Substantial progress along these lines can be expected in the near future, however, especially if the active participants in this quest continue their comparative phylogenetic research. We have no doubt that Professor Schaefer would agree with these sentiments.

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